**PRODUCT INFORMATION AND MANUAL**

Enzyme-linked immunosorbent assay for quantitative detection of rat IFN-γ

For Research Use Only

Not for diagnostic or therapeutic procedures.

96 Wells

# *Rat IFN-γ*

# *Product # 455410*

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# GENERAL INFORMATION

## Product Description

The *Rat IFN-γ ELISA Test Kit* is designed for quantitative determination of the concentration of rat IFN-γ in serum, plasma, and cell culture supernatant.

Rat Interferon gamma (IFN-γ), also known as immune interferon, is a ~15 kDa protein of 137 amino acids (aa) after cleavage of a 19 aa signal peptide. It is presumed that Rat IFN-γ is a noncovalent homodimer. IFN-γ is the only member of the Type II class of interferons. It is produced by activated T, B and NK cells and is an important regulatory cytokine. IFN-γ plays a key role in host defense as it has antiviral, immunoregulatory, and anti-tumor properties. IFN-γ induces the production of cytokines and up-regulates the expression of various membrane proteins including class I and II MHC antigens, Fc receptors, leukocyte adhesion molecules, and B7 antigen. In the mature segment, rat IFN-γ is 87% identical to mouse IFN-γ and 40% identical to human IFN-γ.

The specifications of the *Rat IFN-γ ELISA Test Kit* are as follows:

Format: 96-well strip plate
Assay range: 100-6400 pg/mL
Sensitivity: 100 pg/mL
Total Assay Incubation Time: 3 hours and 45 minutes
Sample Size: 100 μL/well
Sample types: Serum, Plasma and Cell Culture Supernatant
Wavelength for plate reading: 450 nm

## Procedure Overview

The method is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody (capture antibody) specific for rat IFN-γ coated on a 96-well plate. Standards and samples are added to the wells, and any rat IFN-γ present binds to the immobilized antibody. The wells are washed and biotinylated polyclonal anti-rat IFN-γ antibody (detection antibody) is added. After a second wash, avidin-horseradish peroxidase (avidin-HRP) is added, producing an antibody-antigen-antibody sandwich. The wells are again washed and a substrate solution is added, which produces a blue color in direct proportion to the amount of rat IFN-γ present in the initial sample. The stop buffer is then added to terminate the reaction. This results in a color change from blue to yellow. The wells are then read at 450 nm.

## Kit Contents, Storage and Shelf Life

The *Rat IFN-γ ELISA Test Kit* has the capacity for 96 determinations or testing of 40 samples in duplicate (assuming 16 wells for standards and negative controls). Return any unused microwells to the foil bag and reseal them with the desiccant provided in the original package. Store the kit components as recommended in the table below. The shelf life is 12 months when the kit is properly stored.

| Kit Contents | Amount | Storage |
|---|---|---|
| Capture Ab-coated Microtiter Plate | 1 x 96-well plate (8 wells x 12 strips) | 2-8°C |
| Rat IFN-γ Standard | 3 x 30 μL | -20°C |
| 200X Detection Antibody | 75 μL | -20°C |
| 200X Avidin-HRP | 75 μL | -20°C |
| 5X Assay Diluent** | 15 mL | 2-8°C |
| 20X Wash Solution** | 28 mL | 2-8°C |
| Stop Buffer** | 20 mL | 2-8°C |
| TMB Substrate** | 12 mL | 2-8°C |

** Components with the same part numbers are interchangeable among kits as long as they are used before their expiration dates.

## Required Materials Not Provided With the Kit

- Microtiter plate reader (*450 nm*)
- 10, 20, 100 and 1000 μL pipettes
- Multi-channel pipette: 50-300 μL (*Optional*)
- Distilled deionized water

## Sensitivity (Detection Limit)

100 pg/mL

## Specificity (Cross-Reactivity)

| Analytes | Cross-Reactivity (%) |
|---|---|
| Rat IFN-γ: | 100 |
| Human IL-1: | <0.01 |
| Human IL-2: | <0.01 |
| Human IL-4: | <0.01 |
| Human TGF-β: | <0.01 |
| Human IL-5: | <0.01 |
| Mouse IL-1α: | <0.01 |
| Mouse IL-2: | <0.01 |
| Mouse IL-4: | <0.01 |
| Mouse TGF−β: | <0.01 |
| Mouse IL-6: | <0.01 |
| Mouse IL-15 | <0.01 |
| Mouse IL-8: | <0.01 |
| Mouse IL-17: | <0.01 |
| Rat IL-6: | <0.01 |
| Rat TNF-α: | <0.01 |

## Warnings and Precautions

Neogen strongly recommends that you read the following warnings and precautions to ensure your full awareness of ELISA techniques and other details you should pay close attention to when running the assays. More information can also be found in the Troubleshooting section. Periodically, optimizations and revisions are made to the kit and manual. Therefore, it is important to follow the version of the protocol included with the kit. If you need further assistance, please contact a Neogen technical services representative at techservice-lifesciences@neogen.com.

- The standard contains rat IFN-γ. Handle with particular care.
- Do not use the kit past the expiration date.
- Do not intermix reagents from different kits or different lots. ANTIBODIES AND PLATES ARE KIT- AND LOT-SPECIFIC. Make sure that the standards, detection antibody, avidin-HRP, and diluent are mixed in correct volumes.
- Try to maintain a laboratory temperature of (20 – 25°C / 68 – 77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.
- Make sure you are using only distilled deionized water since water quality is very important.
- Incubations of assay plates should be timed as precisely as possible. Be consistent when adding standards to the assay plate. Add your standards first and then your samples.
- Add standards to plate only in the order from low concentration to high concentration as this will minimize the risk of compromising the standard curve.
- Always refrigerate plates in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on plates by allowing them to equilibrate to room temperature (20 – 25°C / 68 – 77°F) before opening (plates provided in packaging contain dessicant).
- Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days. Freeze samples to -20°C or colder if they need to be stored for a longer period. Frozen samples can be thawed at room temperature (20 – 25°C / 68 – 77°F) or in a refrigerator before use.
- Rat IFN-γ in plasma, sera, and cell culture supernatant can be measured directly using this kit without extraction.

# RAT IFN-γ ELISA TEST KIT PROTOCOL

## Reagent Preparation

**IMPORTANT: All reagents should be brought up to room temperature before use (1 – 2 hours at 20 – 25°C / 68 – 77°F). Make sure you read the "Warnings and Precautions" section on page 3.** Solutions should be prepared just prior to ELISA test. All reagents should be mixed by gently inverting or swirling prior to use. Prepare volumes that are needed for the number of wells being run. Do not return the reagents to the original stock tubes/bottles. Disposable reservoirs are recommended to minimize the risk of contamination.

1. **Preparation of 1X Assay Diluent**
   Mix 1 volume of 5X Assay Diluent with 4 volumes of distilled water. In general, 50 mL of 1X Assay Diluent is sufficient for one whole plate.

2. **Preparation of Standards**

   **a.** Add 20 µL of Standard solution to 0.5 mL of 1X Assay Diluent to prepare 6400 pg/mL.

   **b.** Make serial 2X dilutions in the same diluent to make standards of 3200 pg/mL, 1600 pg/mL, 800 pg/mL, 400 pg/mL, 200 pg/mL, and 100 pg/mL. ✐ Discard the remaining unused standards.

   **c.** Use 1X Assay Diluent as negative control.

3. **Preparation of 1X Wash Solution**
   Mix 1 volume of the 20X Wash Solution with 19 volumes of distilled water.

4. **Preparation of 1X Detection Antibody**
   Mix 1 volume of 200X Detection Antibody with 199 volumes of 1X Assay Diluent. When the 200X Detection Antibody is thawed, it can be used for 2 weeks when stored at 4°C. ✐ If you do not plan to use it for a long time, you should store it at a freezer. Repeated freeze-thaw cycles may result in reduced antibody activity.

5. **Preparation of 1X Avidin-HRP**
   Mix 1 volume of 200X Avidin-HRP with 199 volumes of 1X Assay Diluent. When the 200X Avidin-HRP is thawed, it can be used for 2 weeks when stored at 4°C. ✐ If you do not plan to use it for a long time, you should store it at a freezer. Repeated freeze-thaw cycles may result in reduced HRP activity.

## ELISA Testing Protocol

Label the individual strips that will be used and prepare working solutions of reagents as shown in the following example. Adjust the total amount as needed for number of wells that will be used.

| Component | Volume per Well | 24 Wells |
|---|---|---|
| 1X Detection Antibody | 100 µL | 2.4 mL |
| 1X Avidin-HRP | 100 µL | 2.4 mL |
| 1X Wash Solution | 3.0 mL | 72 mL |
| Stop Buffer | 100 µL | 2.4 mL |
| TMB Substrate | 100 µL | 2.4 mL |

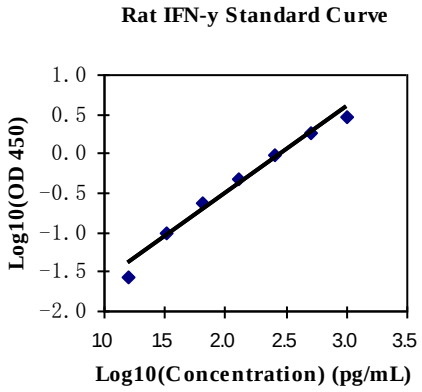
1. Add 100 µL of 1X Assay Diluent to duplicate wells to serve as negative controls. Add 100 µL of each of Rat IFN-γ Standards in duplicate into different wells (✍ *Add standards to plate only in the order from low concentration to high concentration*).
2. Add 100 µL of each sample in duplicate into different sample wells.
3. Incubate for 2 hours at room temperature (*20 – 25°C / 68 – 77°F*) (🛇 ✍ *Avoid direct sunlight and cold bench tops during the incubation. Covering the plate with aluminum foil while incubating is recommended*).
4. Aspirate all fluid from each well after the incubation and wash wells 3 times with 250 µL of 1X Wash Solution per wash. It is not necessary to agitate the plate during the wash steps. Allow Wash Solution to remain in wells for 1-2 min during each wash step. After last wash, invert the plate and gently tap the plate on paper towels to remove residual fluid. (✍ *Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps*).
5. Add 100 µL of 1X Detection Antibody into each well and incubate the plate for 1 hour at room temperature.
6. Aspirate and wash the plate 3 times with 250 µL of 1X Wash Solution per wash. After the last wash, invert the plate and gently tap the plate on paper towels (✍ *Perform the next step immediately after the third wash. Do not allow the plate to air dry between steps*).
7. Add 100 µL of 1X Avidin-HRP into each well. Incubate the plate for 30 minutes at room temperature (*20 – 25°C / 68 – 77°F*).
8. Wash the plate 3 times with 250 µL of 1X Wash Solution per wash. After the last wash, invert the plate and gently tap the plate dry on paper towels (✍ *Perform the next step immediately after the third wash. Do not allow the plate to air dry between steps.*)
9. Add 100 µL of TMB Substrate and incubate for 15 minutes at room temperature (*20 – 25°C / 68 – 77°F*). Start timing the reaction immediately after adding the substrate to the last well. Mix the solution by gently rocking the plate manually during the first minute of incubation. (🛇 ✍ *Do not put any substrate back into the original container which could lead to contamination. Any substrate solution exhibiting coloration is indicative of deterioration and should be discarded. Covering the plate with aluminum foil while incubating is recommended*).
10. Add 100 µL of Stop Buffer in the order of adding TMB substrate to stop the enzyme reaction.
11. Read the plate as soon as possible following the addition of Stop Buffer. Read plate on a

plate reader at 450 nm wavelength (✐ *Before reading, wipe the bottom of the plate with a lint-free tissue to remove any moisture or fingerprints that could interfere with the reading.*)

## Rat IFN-γ Concentration Calculations

A standard curve can be constructed by plotting the adjusted average absorbance for each reference standard against its concentration in pg/mL on a logarithmic scale as shown in the figure below. The adjusted average absorbance is obtained by subtracting the average of the negative control absorbance from the average of each observed absorbance.

To determine the corresponding concentration of rat IFN-γ in pg/mL in samples, plot the adjusted absorbance value for each sample on the standard curve. The figure to the right shows a typical rat IFN-γ standard curve.



# TROUBLESHOOTING

## *No Color Development or No Signals with Standards*

| Possible Causes | Recommended Action |
|---|---|
| *Reagents were used in the wrong order or a step was skipped.* | Follow the protocol carefully and repeat the assay. |
| *Wrong antibodies were used. Either Detection Antibody or Avidin-HRP was prepared incorrectly or has deteriorated.* | Make sure that the antibodies used are the ones that came with the kit. All antibodies are kit- and lot-specific. Make sure that the Detection Antibody, Avidin-HRP and diluent are mixed in correct volumes. |
| *TMB Substrate has deteriorated.* | Use a new set of TMB substrate. Note, if TMB substrate shows any color before use, it should not be used for the assay. |

## *Low Optical Density (OD) Readings*

| Possible Causes | Recommended Action |
|---|---|
| *Reagents were expired or mixed with a different lot number.* | Verify the expiration dates and lot numbers. |
| *Wash solution was prepared incorrectly.* | Verify that the solution was prepared as described in the protocol. |
| *Too many wash cycles were used.* | Make sure to use the number of washes per the protocol instruction. |
| *Incubation times were too short.* | Follow protocol and ensure accurate incubation time. |
| *Lab temperature was too low.* | Maintain the lab room temperature within (20 – 25°C / 68 – 77°F). Do not run assays under air conditioning vents or near cold windows. |
| *Reagents and plates were too cold.* | Make sure that the plates and reagents are brought up to room temperature. |
| *Reader was at wrong wavelength, or reader was malfunctioning.* | Make sure that the wavelength is set to 450 nm and read the plate again. Verify reader calibration and lamp alignment. |
| *Excessive kit stress has occurred.* | Check records to see how many times the kit has cycled from the refrigerator. Check to see if the kit was left at extreme temperatures for too long. |
| *Assay plates were compromised.* | Always refrigerate plates in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on plates by allowing them to equilibrate to room temperature (20 – 25°C / 68 – 77°F) while in the packaging. |

## *High Background or High Optical Density (OD) Readings*

| Possible Causes | Recommended Action |
|---|---|
| *Poor quality water was used in wash solution.* | If water quality is questionable, try substituting an alternate source of distilled deionized water to prepare the wash solution. |
| *Substrate solution has deteriorated.* | Make sure that the substrate is colorless prior to addition to the plate. |
| *There was insufficient washing or poor liquid handling technique.* | Use the number of washes per the protocol instruction. Make sure that 250 µL of wash solution is dispensed per well per wash. If you use a multichannel pipette or robotic liquid handling system, verify its performance; have the system repaired if any ports drip, dispense or aspirate poorly. |
| *Reader was malfunctioning or not blanked properly. This is a high possibility if the OD readings were high and the color was light.* | Verify the reader's performance using a calibration plate and check the lamp alignment. Verify the blanking procedure, if applicable, and reblank. |
| *Lab temperature was too high.* | Maintain the room temperature within (20 – 25°C / 68 – 77°F). Avoid running assays near heat sources or in direct sunlight. |
| *Reagents were intermixed, contaminated or prepared incorrectly.* | Ensure that the correct reagents were used, that working solutions were prepared correctly and that contamination has not occurred. |

## *High Intra-Plate or Inter-Plate Variance*

| Possible Causes | Recommended Action |
|---|---|
| *Inconsistent time was taken when adding standards, reagents or samples within and/or between plates.* | Make sure that all materials are set up and ready to use. Use a multichannel pipette to add reagents to multiple wells whenever possible. Do not interrupt procedure while adding standards, reagents and samples. |
| *Multichannel pipette was not functioning properly.* | Verify pipette calibration and check that tips are on tight. Be sure all channels of the pipette draw and dispense equal volumes. |
| *There was inconsistent washing or poor liquid handling technique.* | Use the number of washes per the protocol instruction. Make sure that 250 µL of wash solution is dispensed per well per wash. If you use a multichannel pipette or robotic liquid handling system, verify its performance; have the system repaired if any ports drip, dispense or aspirate poorly. |
| *Inconsistent incubation times occurred from plate to plate.* | Time each plate separately to ensure consistent incubation times. |
| *Pipette was inaccurate.* | Check the pipette calibration. Verify that pipette tips are on tight before use and that all channels draw and dispense equal volumes. |
| *Kit plates, reagents, standards and samples were at different temperatures.* | Make sure to allow sufficient time for kit plates, reagents, standards and samples to come to room temperature (20 – 25°C / 68 – 77°F). Larger volumes will require longer equilibration time. If using a water bath to hasten equilibration, make sure that it is maintained at room temperature; do not use a warm water bath to warm reagents, samples and kit standards. |
| *Reagents used were intermixed from different kit lots, or the kits were of different expiration dates.* | Carefully label each user-prepared reagent to make sure that the reagents are not intermixed. Kits with different expiration dates might generate different range of OD readings, however, the relative absorbance values will typically be comparable. In general, a value of less than 1.0 reading for the highest standard may indicate deterioration of reagents. |

## *One or More of the Standard Curve Points Are Out of Range*

| Possible Causes | Recommended Action |
|---|---|
| *Standards were added in wrong order or recorded in wrong position.* | Make sure that the standards are applied and recorded correctly. |
| *Standards were contaminated or intermixed with other standards.* | Prepare a new set of standards. Always add standards to plate in the order from low concentration to high concentration. |
| *There was inconsistent washing or poor liquid handling technique.* | Use the number of washes per the protocol instruction. Make sure that 250 µL of wash solution is dispensed per well per wash. If you use a multichannel pipette or robotic liquid handling system, verify its performance; have the system repaired if any ports drip, dispense or aspirate poorly. |
| *Inconsistent time was taken to add standards and reagents to plate.* | Make sure all materials are set up and ready to use. Add standards to plate only in the order from low concentration to high concentration at undisrupted constant pace. Use a multichannel pipette to add reagents to multiple wells simultaneously to increase consistency. |
| *Multichannel pipette was not functioning properly.* | Verify pipette calibration and check that tips are on tight. Be sure all channels of the pipette draw and dispense equal volumes. |

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D455410-3/12/12